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RESEARCH ARTICLE
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The Effect of Drying Temperature of *Carica pusbecens* Seeds on Secondary Metabolite

Elok Widayanti[1(CA)], Hanandayu Widwiastuti[2], Rany Adelina[3], Indah Cahyaning Wulan[4]
[1(CA)]Study Program of D3 Pharmacy and Food Analysis, Department of Nutrition, Poltekkes Kemenkes Malang, Indonesia; elok_widayanti@poltekkes-malang.ac.id (Corresponding Author)
[2]Study Program of D3 Pharmacy and Food Analysis, Department of Nutrition, Poltekkes Kemenkes Malang, Indonesia; hanandayu_widwiastuti@poltekkes-malang.ac.id
[3]Bachelor Program in Applied Nutrition and Dietetics, Department of Nutrition, Poltekkes Kemenkes Malang, Indonesia; rany.adelina@gmail.com
[4]Study Program of D3 Pharmacy and Food Analysis, Department of Nutrition, Poltekkes Kemenkes Malang, Indonesia; cahyaningwulanindah@gmail.com

ABSTRACT

Papaya mountain (*Carica pubescens*) is one of the typical plants that grows in the Dieng Plateau. Seeds of mountain papaya (*Carica pubescens*) are a by-product of candied carica production which has not been widely used. All carica seeds obtained were cleaned of mucilage and then treated with drying temperatures of 30, 40, 50 and 60ºC. The purpose of this study was to examine the secondary metabolite content of the ethanol extract of mountain papaya seeds with variations in drying temperature. The type of research used was experimental. Drying temperature was one of the factors that affect the quality of simplicia. The extraction method used maceration with 70% ethanol as a solvent. Maceration was carried out for 3 days followed by a phytochemical screening test. The results of the phytochemical test showed that the ethanol extract of papaya seeds at temperatures of 30, 40, 50 and 60oC contained alkaloids, flavonoids, saponins, tannins and triterpenoids.

Keywords : *Carica pubescens*; temperature variations; maceration

INTRODUCTION

Dieng Plateau is located in Central Java Province with an average elevation of around 2000 meters above sea level. The Dieng area has a unique plant that can thrive, namely mountain papaya (*Carica pubescens*). This mountain papaya is still in the same genus as papaya (*Carica papaya*) with the species names pubescens, Lenne, and Koch[(1)].The head of the Carica Craftsmen Association (APC) group in the Wonosobo area stated that the carica candied production process produces carica seed waste weighing an average of 9 tons/month. The waste generated is a separate problem because it requires a large enough disposal site. So far, there is no special storage place for this waste. An assessment of carica seed waste can be carried out in order to provide insight into where it is hoped that the use of carica seed waste will be a breakthrough that can be made by the community so that it can increase its economic value. This increase is expected to be accompanied by an increase in the economic level of the community. For this reason, it is necessary to carry out further studies of carica seed waste so that the potential of carica seeds is not wasted [(2)]. In a study conducted by Minarno (2015) it was stated that the results of qualitative tests of *Carica pubescens* fruit growing in the Cangar, Bromo and Dieng Plateau areas through phytochemical screening contained flavonoids, polyphenols, tannins and triterpenoids [(3)]. In addition, Rina Wijayanti's research stated that *Carica pubescens* seed extract contains alkaloids, flavonoids, phenols, saponins, tannins and terpenoids [(4)].

In previous studies, the effect of drying temperature on the phytochemical content of *Carica pubescens* seeds had not been disclosed. According to Mulia Syafrida's research, it was stated that the higher the drying temperature, the lower the level of flavonoids [(5)]. The proper drying process can maintain the continuity of the active ingredients contained in the simplicia. Thus, the content of the active ingredients is not lost and the quality of the simplicia can be maintained [(6)].

Based on the above background, it is necessary to conduct research that aims to determine the effect of drying temperature of *Carica pusbecens* seeds on secondary metabolite content. With this research, it is hoped that it can provide knowledge regarding the chemical content of *Carica pubescens* seeds.

METHODS

Drying and Extraction

Carica pubescens seeds were collected from ripe fruit from Dieng Plateau, Wonosobo Regency. Carica seeds were washed with running water repeatedly, dried using an oven with variations in drying temperature, namely 30, 40, 50, and 60° C.

The dried carica seeds were then ground using a grinder and sieved using mesh number 60. Carica seed powder was extracted using the maceration method using 70% ethanol solvent with a ratio of materials and solvents, namely 1:10. Maceration was carried out for 3x24 hours with occasional stirring. The filtrate obtained is then evaporated by the solvent using a water bath until a thick extract is obtained.

Alkaloid Screening

The ethanol extract of *Carica pusbecens* seeds was taken as much as 0.25 gram and added five mL of 2N HCl solution. Heated using a hotplate for two minutes, cooled, and filtered. The filtrate was divided into three different test tubes, one tube as a blank. The other two tubes were added three drops each of Dragendorf's and Mayer's reagent. A positive alkaloid result is indicated by the appearance of an orange precipitate in the second tube and a white or yellowish precipitate in the third tube [(7)].

Flavonoid Screening

The ethanol extract of *Carica pusbecens* seeds was added with 96% ethanol until dissolved. Add 3 drops of concentrated HCl solution slowly through the test tube wall. Added magnesium powder into the solution. A positive result for flavonoids is indicated by a change in the color of the solution to orange [(8)].

Saponin Screening

The ethanol extract of *Carica pusbecens* seeds was added to 10 mL of hot water and then waited until the temperature was normal. Do vigorous shaking for 10 seconds. The test result is positive for saponins when a firm froth is formed for 10 minutes with a height of 1-10 cm. Added 2N HCl solution and the foam did not disappear [(9)].

Tannin Screening

The ethanol extract of Carica pusbecens seeds was added with 1 mL of 10% FeCL3 solution. A positive test result for tannins is when the solution changes to a dark blue, blackish blue, or greenish black color [(7)].

Triterpenoid Screening

The ethanol extract of Carica pusbecens seeds was dissolved in 0.5 mL of chloroform solution. Added with 0.5 mL of anhydrous acetic acid solution. The solution was added with 2 mL of H2SO4. The positive test result for triterpenoids is the formation of a brownish or violet ring [(7)].

RESULT

Phytochemical Screening of Ethanol Extract of Carica pubescens Seeds

Table 1. Results of phytochemical screening of ethanol extract of *Carica pubescens* seeds

Sample temperature	Phytochemical Screening				
	Alkaloid	Flavonoid	Saponin	Tannin	Triterpenoid
30°C	+	+	+	+	+
40°C	+	+	+	+	+
50°C	+	+	+	+	+
60°C	+	+	+	+	+

Note + : positive for secondary metabolites

Phytochemical screening uses a qualitative test method based on the color change reaction and precipitation reaction. The purpose of this phytochemical screening is to describe the content of secondary metabolites in carica seed extract samples. Screening results are shown in the following table:

Alkaloid

A total of 0.25 grams of ethanol extract of carica with a drying temperature of 30oC was added with 5 mL of 2N HCl solution and then heated using a hotplate for 2 minutes. The addition of HCl is because the alkaloids have basic properties so they are extracted using an acidic solvent [8]. Furthermore, carica seed extract when dripped with Dragendorf and Meyer reagents. When Dragendorf's reagent was added, an orange precipitate was obtained. This precipitate is thought to be a potassium-alkaloid. This shows that the seed extract of *Carica pubescens* positively contains alkaloids [10]. And when Meyer's reagent is added it produces a yellow precipitate, this also shows a positive result containing alkaloids. The same treatment was carried out on samples of ethanol extract with drying temperatures of 40, 50 and 60ºC. The results obtained were positive for alkaloids.

Flavonoid

In the ethanol extract of Carica pusbecens seeds with a drying temperature of 30ºC, 96% ethanol was added to dissolve the extract. Then added concentrated HCl solution and magnesium powder into the solution and produced an orange color. This orange color is formed due to the formation of flavone compounds. Furthermore, the same treatment was carried out for samples with drying temperatures of 40, 50 and 60ºC. The results obtained were positive for flavonoids.

Saponin

The ethanol extract of *Carica pusbecens* seeds at a drying temperature of 30ºC was added to 10 mL of hot water and then waited until the temperature was normal. Then vigorous shaking was carried out for 10 seconds. The results of the saponin test on carica pubescens seed extract showed positive saponins due to the formation of foam which did not disappear for 10 minutes. The appearance of foam in water is due to the presence of glycosides which hydrolyze into glucose and other compounds [10]. In the same treatment for drying temperatures of 40, 50 and 60ºC, the results were the same, namely positive for saponins.

Tannin

The ethanol extract of Carica pubescens seeds at a drying temperature of 30ºC was added with 1 mL of 10% $FeCl_3$ solution. After the addition of $FeCl_3$, the solution changed to a dark blue color. It is possible for a reaction to occur between one of the hydroxyl groups in the tannin compound and $FeCl_3$. At drying temperatures of 40, 50 and 60ºC the same results were also obtained, namely positive tannins.

Triterpenoid

The results of the phytochemical screening showed that the ethanol extract of Carica pusbecens seeds with a positive drying temperature of 30oC contained triterpenoids marked by the formation of brown rings after adding 2 mL of concentrated H2SO4 through the tube wall. The same treatment was carried out on samples with drying temperatures of 40°C, 50°C and 60°C and showed positive results for each drying temperature treatment [3].

DISCUSSION

Carica fruit that has been obtained is then peeled and separated between the pulp and seeds. Seeds that have been separated then cleaned with running water and in the wind right. The drying method is an important step in simplicia preparation so that it can be used in the next analysis stage. The seeds are placed in the oven to dry. The temperatures used for drying were 30, 40, 50 and 60ºC for 7 hours. This drying temperature variation is carried out using an oven so that it is hoped that the drying process can run faster and stability of the simplicia mass can be achieved immediately. Oven drying is considered more profitable because a large amount of water content will be reduced in a short time [11], besides that oven drying is also the best method with the lowest water content [12]. The use of temperatures that are too high can result in biochemical changes that reduce the quality of the resulting product [13]. So that the temperature variations used are not more than 60ºC, namely between 30-60ºC.

The using of maceration extraction method is to extract the compounds contained in the simplicia using a solvent. The advantage of the maceration method is that the procedure and equipment are simple and do not use

heating so that the secondary metabolite content is not expected to change. Bioactive compounds such as flavonoids, phenols and tannins can be damaged at temperatures above 50°C which can change their molecular structure. Choosing the right extraction method affects the success rate of making the extract [(14)]. Maceration extraction was carried out for 3 x 24 hours using 70% ethanol solvent. The choice of solvent can affect the extraction results. Ethanol is known to be a fairly good solvent used for extraction and can dissolve compounds from less polar to polar, one of the compounds that can be dissolved by ethanol is phenolic compounds. Ethanol can dissolve phenolic compounds because it is capable of degrading cell walls so that bioactive compounds are more easily excreted from plant cells [(15)].

After obtaining the carica seed extract, chemical screening was carried out, namely the alkaloid test. In the alkaloid test, the extract was added with HCl to attract the alkaloid compounds in the extract. The alkaloids in the extract have alkaline properties so they can form salts with the addition of acid [(8)]. Furthermore, carica seed extract when dripped with Dragendorf and Meyer reagents. When Dragendorf's reagent was added, an orange precipitate was obtained. This precipitate is thought to be a potassium-alkaloid. This shows that the seed extract of *Carica pubescens* positively contains alkaloids [(10)]. And when Meyer's reagent is added it produces a yellow precipitate, this also shows a positive result containing alkaloids. The same treatment was carried out on the ethanol extract samples with drying temperatures of 30, 40, 50 and 60ºC. The results obtained were positive for alkaloids.

Flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. Flavonoid testing was carried out by adding concentrated HCl and magnesium metal. The addition of this concentrated HCl solution aims to reduce the benzopirone nucleus contained in the flavonoid structure so that a flavilium salt is formed which makes the solution color orange. Ethanol extract samples with drying temperatures of 30, 40, 50 and 60ºC were given the same treatment. And the results of the solution turned orange. This shows that carica seed extract positively contains flavonoids.

Saponin testing was carried out by adding water and then shaking vigorously for 10 seconds. The results of the saponin test on carica pubescens seed extract showed positive saponins due to the formation of foam which did not disappear for 10 minutes. The emergence of foam in water due to the hydrolyzed glycosides into glucose and other compounds. This is in accordance with research conducted by Marliana et al that in the saponin test foam was formed due to the presence of hydrolyzed glycosides [(10)].

Tannin is one of the polyphenolic compounds which can be tested using FeCl3. The addition of FeCl3 to the carica extract solution causes the extract solution to turn dark blue. This change is caused because tannins will form complex compounds with Fe3+ ions. The Fe3+ ion acts as the central atom and tannins have an O atom which has a lone pair of electrons that can coordinate to the central atom as a ligand [(16)].

Triterpenoids are nonpolar compounds, so in the phytochemical screening test, chloroform is needed as a solvent because they have the same polarity (nonpolar). then anhydrous acetic acid is added to form the acetyl derivative in chloroform. The addition of concentrated H2SO4 through the wall of the test tube causes a reaction between acetic anhydride and acid so that the C atom in the anhydride forms a carbocation. The carbocation formed reacts with the O atom in the –OH group present in the triterpenoid compound. This reaction is an esterification reaction, namely the formation of ester compounds by triterpenoid compounds with acetic anhydride. This can be proven by the formation of a brownish or violet ring at the boundary of the two solvents which indicates a triterpenoid compound [(17)].

CONCLUSION

Based on the results of the phytochemical screening test in this study, it can be concluded that the ethanol extract of Carica pubescens seeds grown in the Dieng plateau at drying temperatures of 30, 40, 50 and 60ºC positively contained alkaloids, flavonoids, saponins, tannins and triterpenoids. Variations in drying temperature did not affect the type of secondary metabolite content of carica pubescens seed extract.

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